

## AMPOULE ANALYZER APPARATUS

This application is a continuation-in-part of both U.S. <sup>BEISNER, 435/303.1</sup> Serial No. 09/557,653, filed April 25, 2000, and U.S. Serial No. <sup>ROSENBERG</sup> 09/578,323, entitled "Light Analyzer Apparatus" and filed May 24, 2000, which are both hereby incorporated by reference herein in their entireties.

## BACKGROUND OF THE INVENTION

## 1. Field of the Invention

This invention relates broadly to analytical instruments. More particularly, this invention relates to an analyzer apparatus for determining when a photometric change occurs in a sample, and relating the change to a condition of the sample at a previous time.

## 2. State of the Art

Water quality, and particularly bacterial content in water is of great concern. Municipalities perform periodic checks of water quality to ensure that water reserves are safe. Recently, home monitoring of water quality has become popular.

A number of products are available for testing water quality. One variety of product is an ampoule which is a sealed evacuated

1 vial containing a powdered nutrient for microbes and an indicator  
2 which changes color when the concentration of microbes reaches a  
3 specific high level. One such ampoule is shown in U.S. Patent No.  
4 5,935,799 to Isbister. In use, a user inverts the ampoule in a  
5 cup of sample water and breaks off a scored tip of the ampoule.  
6 The vacuum in the ampoule causes the ampoule to fill with sample  
7 water. The ampoule is then shaken to mix the powdered nutrient  
8 and indicator with the water. Finally, the ampoule must be  
9 maintained at a constant temperature, e.g., 34°C, for a relatively  
10 long period of time, e.g., up to 12 hours. One manner often  
11 suggested by manufacturers of the ampoules for maintaining proper  
12 temperature is for the user to place the ampoule in a shirt pocket  
13 of the user, since the shirt pocket is approximately at the  
14 desired temperature. Periodically, e.g., every thirty minutes,  
15 the user must look for the indicator to change color by holding  
16 the ampoule up the light and comparing the observed color against  
17 a printed chart. When the color change is observed, the elapsed  
18 time is recorded and a second chart is used to look up the number  
19 of microbes that were in the original sample water based upon the  
20 recorded time.

21  
22 The determination of the number of microbes is based on the  
23 fact that microbes multiply by binary fission. The number of  
24 microbes in the original sample may therefore be determined by  
25 reference to an exponential chart and the recorded time.

1 While this type of analytical product is useful, it has  
2 several drawbacks. One problem is the requirement to hold the  
3 ampoule in a shirt pocket for incubation. Another problem is that  
4 the accuracy is questionable, as human judgment is required to  
5 read the color at the end point.

6  
7 Several apparatus have been disclosed to test samples, but  
8 they do not address the testing requirements for the above  
9 described ampoules. For example, U.S. Patent No. 5,013,155 to  
10 Rybak discloses an apparatus which determines a specific color of  
11 a sample in a vial received in a receptacle in the apparatus. The  
12 device uses two light sources, each of a different color, which  
13 are alternately pulsed, and respective photodetectors. The  
14 results are interpolated along with signals present when no light  
15 is emitted, to identify the specific color of the test sample.  
16 The Rybak apparatus requires a vial of clear distilled water to  
17 calibrate the instrument. In addition, the device is not adapted  
18 for heating test vials at a constant temperature.

19  
20 U.S. Patent No. 5,959,738 to Hafeman et al. uses either a  
21 single light source capable of operating at multiple wavelengths,  
22 or multiple and different wavelength light sources. A  
23 relationship is determined between the light absorption properties  
24 of a liquid sample (solvent and analyte) and the optical  
25 pathlength of the liquid sample to calculate a concentration of an



1        It is a also an object of the invention to provide an  
2 apparatus which determines at time at which a targeted photometric  
3 change occurs in an sample, and relating the targeted change to a  
4 condition of the sample at a previous time.

5  
6        It is yet another object of the invention to provide an  
7 apparatus which maintains ampoules at a desired temperature.

8  
9        It is yet a further object of the invention to provide a  
10 portable and relatively low cost apparatus for heating and  
11 analyzing changes in the contents of an ampoule.

12  
13       In accord with these objects, which will be discussed in  
14 detail below, an ampoule analyzer apparatus is provided which  
15 includes a housing having at least one receptacle (or nest) for an  
16 ampoule, a cover for substantially preventing ambient temperature  
17 and light from affecting each receptacle, a light analysis system,  
18 an incubation system, and a master control system.

19  
20       The light analysis system includes, for each receptacle, at  
21 least one light source and a photodetector positioned such that  
22 the light from the light source passes through the receptacle (and  
23 thereby the ampoule and its contents) prior to entering the  
24 photodetector. The light source is chosen to deliver a  
25 predetermined wavelength of light such that the color change of

1 the contents of the ampoule causes reduction in the intensity of  
2 the light transmitted through the contents of the ampoule.

3  
4 The incubation system includes, for each receptacle, a  
5 heating element which rapidly heats the receptacle to a desired  
6 temperature and a temperature sensor which senses the temperature  
7 of the receptacle. Each receptacle is preferably insulated to  
8 prevent unintended heating of neighboring receptacles of the  
9 apparatus.

10  
11 The master control system permits user input, operates the  
12 light analysis system and the incubation system. In addition, the  
13 master control system includes a timer, and a memory provided with  
14 a look-up table relating the type of test, the time to test  
15 completion, and the associated bacterial count at the start of the  
16 test. A user-readable display for the output of the results,  
17 e.g., the bacterial count at time zero, is also provided.

18  
19 During operation, an ampoule containing a water sample and a  
20 reagent which causes the sample to change color when a certain  
21 level of biological activity is present in the sample is placed  
22 within one of the receptacles. The light analysis system is  
23 operated to transmit light at the predetermined wavelength through  
24 the ampoule (either axially or transversely) to the detector, and  
25 a maximum amount (intensity) of light passing through the ampoule

1 is determined. The incubation system is also operated to heat the  
2 receptacle and the ampoule therein to a desired test temperature  
3 and the timer is started. The light analysis system periodically  
4 transmits light through the ampoule. Increased biological  
5 activity in the sample causes a color change to the indicator  
6 which reduces light transmission through the ampoule. When the  
7 light detected at the detector is reduced relative to the light  
8 transmitted by a predetermined percentage of the maximum amount of  
9 light, the master control system signals that the test is  
10 complete. Based on the amount of time required for this to occur,  
11 the master control system determines from the look-up table the  
12 bacterial content in the sample at the beginning of the test and  
13 displays the results on the display.

14  
15 The apparatus may include a large number of receptacles  
16 suitable for laboratory use or may include fewer or one receptacle  
17 suitable for home or portable use.

18  
19 Additional objects and advantages of the invention will  
20 become apparent to those skilled in the art upon reference to the  
21 detailed description taken in conjunction with the provided  
22 figures.

23

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a schematic circuit diagram of the apparatus of the invention;

Fig. 2 is a partial side view of the apparatus of the invention showing the case lid in open and closed positions;

Fig. 3 is a partial front view of the analyzer apparatus of the invention;

Fig. 4 is a top view of the apparatus of the invention without the case lid;

Fig. 5 is a side view of an ampoule receptacle according to the invention;

Fig. 6 is a front view of an ampoule receptacle according to the invention; and

Fig. 7 is a flow chart illustrating the operation of the apparatus of the invention.



## 1 DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

2  
3 Turning now to Figs. 1 through 4, an ampoule analyzer  
4 apparatus 10 according to the invention includes a housing 12  
5 having a light analysis system (a light source 14 and an optical  
6 detector 16, collectively ), an incubation system (a heating  
7 element 18 and a sensor chip 19, collectively), and a master  
8 control system 20, each of which is discussed in detail below.  
9 The housing 12 also includes a battery 24 and associated circuitry  
10 26 to power the various systems. A preferred battery 24 and  
11 circuitry 26 are disclosed in previously incorporated U.S. Serial  
12 No. 09/578,323.

13  
14 Six receptacles (nests) 30, each for receiving an ampoule 32,  
15 are provided in the housing. The housing 12 is also preferably  
16 provided with a planar lower surface 34 which is adapted to seat  
17 the housing on a planar surface, and a storage area 36 for storing  
18 ampoules or other items. A lid 38 movable between closed and open  
19 (broken lines) positions covers and uncovers the receptacles 30,  
20 the storage area 36, and other exposed components to protect them  
21 from the elements, and to facilitate transportation of the  
22 apparatus.

1 A receptacle cover 40 in an open position provides access to  
2 the receptacles 30 and in a closed position 40a substantially  
3 individually seals each receptacle to prevent ambient light from  
4 affecting the receptacle. The receptacle cover 40 preferably  
5 includes a plurality of concave portions 42 each having a diffuse  
6 reflective interior surface 44 which reflects and distributes  
7 light from a light source, discussed below, through the  
8 receptacles.

9  
10 Each receptacle 30 is an opaque tube; e.g., metal or plastic,  
11 approximately 0.5 - 0.625 inch in diameter, and preferably  
12 approximately four inches in length. The receptacles 30 are  
13 individually surrounded by a thermally insulative foam 46 to  
14 maintain the temperature of the receptacle during heating by the  
15 incubation system, as described below. Turning to Figs. 5 and 6,  
16 a transparent preferably cleanable disk 48, preferably glass or  
17 polycarbonate, is provided near a lower end 30a of the receptacle.  
18 The closed end 32a of the ampoule 32 is provided near the disk 48,  
19 with the open end 32b of the ampoule at the upper end 30b of the  
20 receptacle (Fig. 2). An O-ring 50 provides a watertight seal  
21 between the disk 48 and the interior surface of the receptacle 30.  
22 A weep hole 51 is provided in the receptacle adjacent but above  
23 the location of the disk 48 to permit any water, test solution, or  
24 cleaning/sterilization solution which may drip into the receptacle  
25 30 to drain therefrom.

Referring to Figs. 1, 2 and 5, the receptacles 30 are attached to a printed circuit board (PCB) 52, e.g., by screws 54, preferably such that a longitudinal axis of each receptacle runs parallel to the plane of the PCB. Each receptacle 30 is provided with the light source 14 and the optical detector 16 which are each electrically coupled to a microcontroller 60 of the master controller 20. Both the light source 14 and optical detector 16 are also preferably physically coupled to the PCB 52. The light source 14 includes one or more LEDs adapted to emit light at a predetermined wavelength into the receptacle 30 when receiving a signal 54 from the microcontroller 60. According to a preferred embodiment of the invention, the light source 14 is a plurality of LEDs coupled to the PCB 52 in an orientation such that they preferably direct light into the reflective interior surface 44 of the associated portion 42 of the cover 40 of housing 12. The reflective surface 44 scatters the light of the LEDs 14 substantially axially through the ampoule 32 in the receptacle 30 and toward the detector 16 located at the lower end 30a of the receptacle (Figs. 1 and 2). A preferably hemispherical lens 56 is preferably provided to gather the scattered light and channel the light transmitted through the ampoule 32 toward the detector 16 (Figs. 5 and 6). The optical detector 16 provides a return signal 58 to the microcontroller 60. The return signal 58 is amplified and filtered with a time constant to null out any short term changes which may be caused by bubbles breaking at the top

1 surface. The analog return signal is provided to an analog to  
2 digital converter associated with the microcontroller 60.

3  
4 The receptacles 30 are preferably provided at an oblique,  
5 non-perpendicular angle relative to both the vertical and the  
6 horizontal, e.g., 30° to 45° off vertical, by angling the  
7 receptacles relative to the lower surface 34 of the housing 12.  
8 The angle of the receptacles 30 facilitates axial light  
9 transmission through the ampoules by preventing sediment from  
10 accumulating on the entire bottom of the ampoule and thereby  
11 blocking all light paths between the reflective surface 44 and the  
12 optical detector 16. Moreover, the ampoules are preferably  
13 provided with a stirring rod which will settle outside a direct  
14 axial light path when the receptacles are angled. As the  
15 receptacles 30 are preferably coupled to the PCB 52, one preferred  
16 manner of providing the angle is to orient the entire PCB at the  
17 desired angle relative to vertical within the housing 12. The  
18 above described configuration of the light source 14, optical  
19 detector 16, and orientation of the receptacles 30 provides a  
20 system in which all componentry is preferably provided at or below  
21 the level of the top 32b of the ampoule 32. This configuration  
22 facilitates sealing the receptacles 30 from ambient light, with  
23 the reflective surface 44 of the cover 40 providing the  
24 redirection of the light into the required path through the  
25 receptacle and ampoule. In addition, as the cover 40 is capable

1 of reflecting the light, the need for separate reflectors is  
2 obviated and a system with fewer components, and therefore lower  
3 cost, is provided.

4  
5 As briefly discussed above, the light source 14, preferably a  
6 plurality of LEDs, is adapted to emit light at a predetermined  
7 wavelength. Optionally, the LEDs may emit light at different  
8 wavelengths, and then, depending upon which wavelength is desired,  
9 the LED which produces light at that wavelength is selected. In  
10 addition, the master control system 20 may be operated to cause  
11 the microcontroller 60 to signal all the LEDs of the light source  
12 14 to emit light constantly, alternately, or to be pulsed.

13  
14 Still referring to Figs. 1, 2 and 5, the incubation system  
15 includes the heating element 18 adapted to heat the receptacle 30  
16 and a temperature sensor chip 19 in contact with the receptacle 30  
17 for determining the temperature thereof. The heating element 18  
18 includes a pack of heating resistors 66 and a driver FET (field  
19 effect transistor) 68 which is also coupled to the microcontroller  
20 60 of the master control system 20. The temperature sensor chip  
21 19 is preferably a silicon device which produces a voltage related  
22 to a sensed temperature. The sensor chip 19 is preferably held  
23 tightly against the receptacle 30 to accurately sense the  
24 temperature of the receptacle. Preferably, one of the screws 54  
25 provides a heat conductive path from the receptacle 30 to the

1 temperature sensor 19, and a tie wrap 70 preferably sandwiches the  
2 heating resistors 66 between the PCB 42 and the receptacle 14  
3 (Fig. 5). The incubation system is preferably calibrated to  
4 quickly and accurately heat the receptacle (and consequently the  
5 ampoule provided therein) to a desired temperature. Calibration  
6 of the incubation system, as well as software control and  
7 associated control signals 72, 74 to and from the heating element  
8 18 and sensor chip 19 to bring and maintain the receptacle 30 to a  
9 desired temperature, are discussed in detail in co-pending and  
10 previously incorporated U.S. Serial No. 09/557,653.

11  
12 Referring back to Fig. 1, the master control system 20 of the  
13 analyzer apparatus 10 includes the microcontroller 60, a timer,  
14 and a control button 76 permitting user input and operation. In  
15 addition, the master controller 20, through the microcontroller 60  
16 operates the light analysis system 14, 16 and the incubation  
17 system 18, 19, and provides information to a user-readable display  
18 80 and a signal 82 to an audio output 84 (comprised of a driver  
19 chip 86 and a sound transducer 88) for the output of the results  
20 of testing with the analyzer.

21  
22 The ampoules 32 used in the apparatus, the operation of which  
23 is discussed below, contain a water sample and a reagent which  
24 changes color (a color indicator) when a certain level of  
25 biological activity (total microbial count, E. Coli, Coliform,

1 etc.) is present. Numerous such reagents are disclosed in detail  
2 in U.S. Patent Nos. 4,204,037 to Frosch et al., 4,332,769 to Rampy  
3 et al., 5,212,876 to Turner et al., and 5,935,799 to Isbister,  
4 each of which is hereby incorporated by reference herein in its  
5 entirety. For each type of ampoule, an ampoule calibration is  
6 performed to determine the percentage of light reduction which  
7 occurs at a particular light wavelength when the indicator turns  
8 sufficient color to indicate an end of test. For example, an  
9 ampoule containing a reagent used to indicate the total microbial  
10 count in a sample has been shown to reduce light transmission to  
11 seventy-five percent of the maximum light transmission through a  
12 sample by its color change at test end. The types of ampoules,  
13 and the predetermined amount of light transmission reduction at  
14 particular light wavelengths required to indicate test completion  
15 is stored in a memory of the master control system 20.

16  
17 Turning now to Fig. 1, 2 and 7, in operation, an ampoule 32  
18 is placed at 100 in a receptacle 30 of the apparatus 10. The  
19 master controller is operated at 102 to indicate a type of  
20 ampoule; i.e., reagent and test being performed. The incubation  
21 system is then activated at 104 to begin bringing the ampoule to  
22 the desired test temperature, e.g., 34 °C, which increases the  
23 biological activity in the sample. In addition, the light  
24 analysis system is operated at 106 to transmit light at a  
25 predetermined wavelength, selected for the ampoule under test,

1 through the ampoule to the detector. The light level (intensity)  
2 measured by the photodetector and logged at 108 at regular  
3 intervals, e.g., every minute. The light transmission through the  
4 ampoule may initially increase, as small bubbles rise out and  
5 larger bubbles break at the surface. Once the light level stops  
6 rising at 110, the light level is logged and indicated at 112 as  
7 the maximum amount of light transmission that can be expected  
8 through the ampoule. This self-calibration test is carried out  
9 for each ampoule in each receptacle.

10  
11 Meanwhile, the temperature sensor chip 19 reads at 114 the  
12 temperature of the receptacle. When the chip 19 senses at 116  
13 that the temperature of the ampoule is close to the target  
14 temperature, e.g., within one to five degrees Celsius of the  
15 target temperature, the timer is activated at 118 to begin  
16 counting time until the indicator changes color sufficient to  
17 reduce the light transmission to a predetermined percentage of the  
18 maximum.

19  
20 The detection of the indicator color changes is enhanced by  
21 the choice of the light source wavelength. For example, an  
22 ampoule for testing the total microbial count turns red as the  
23 microbial count rises. As such, 565 nm green LEDs are preferred  
24 for light transmission through the ampoule in such a test, as the  
25 red reagent indicator effectively limits transmission of 565 nm



1 light therethrough. The LED color (i.e., light wavelength) is  
2 selected by the master control system 20 based upon the type of  
3 ampoule selected. In addition, as the sample may contain some  
4 degree of turbidity; i.e., debris or small air bubbles that will  
5 scatter light, it has been found that the preferred green  
6 wavelength is relatively insensitive to the light scattering  
7 effects of turbidity. Furthermore, as stated above, the effect of  
8 turbidity is also limited by the initial self-calibration.

9  
10 Periodically, e.g., every minute to every hour, light is  
11 transmitted at 120 by the light source through the ampoule. This  
12 continues at 122 until the color change in the reagent is  
13 sufficient to reduce at 124 light transmission of the selected  
14 wavelength by a predetermined amount and indicates the test is  
15 complete at 126. The timer is then stopped at 128. It is noted  
16 that at no time during the test is a human visual comparison  
17 between the color of the contents of the ampoule and a reference  
18 required. Based on the amount of time required for test  
19 completion, the master control system 20 determines at 130 from a  
20 look-up table stored in memory the bacterial content in the sample  
21 at the beginning of the test and displays at 132 the results on  
22 the display 80. The result is preferably displayed until a new  
23 test is started or the power switch is turned off.

24

1 According to a second embodiment of the apparatus,  
2 substantially similar to the first, the light source and light  
3 detector are located on opposite sides of the receptacle rather  
4 than at the ends thereof. As such, light is transmitted  
5 transaxially across the receptacle and through the ampoule. The  
6 apparatus of the second embodiment is used in substantially the  
7 same manner as the first embodiment. However, it is noted that  
8 the samples in some ampoules under test may contain microbes that  
9 form various films at different levels within the ampoule; i.e., a  
10 stratification of the sample. As such, the axial embodiment is  
11 preferred as it eliminates any artifacts caused by stratified  
12 layers. In addition, the axial measurement embodiment permits the  
13 light to be transmitted through about four inches of the sample  
14 water, as opposed to about 0.5 inch of water with the transaxial  
15 mode. The larger amount of sample water provides a proportionally  
16 denser color change when the indicator changes color.

17  
18 There have been described and illustrated herein embodiments  
19 of an analyzer apparatus. While particular embodiments of the  
20 invention have been described, it is not intended that the  
21 invention be limited thereto, as it is intended that the invention  
22 be as broad in scope as the art will allow and that the  
23 specification be read likewise. Thus, while a battery and  
24 associated circuitry have been disclosed, it will be appreciated  
25 that other power systems may be used as well. In addition, while

1 a particular calibration system has been disclosed for the  
2 incubation system, other incubator calibration systems may be used  
3 as well. In addition, while the temperature sensor is described  
4 as producing a voltage proportional to a sensed temperature, it  
5 may alternatively produce a voltage inversely proportional to a  
6 sensed voltage, each of which is considered 'proportional' in the  
7 claims. Also, while a field effect transistor is preferred as  
8 part of the heating element, other transistors, such as a  
9 switching transistor, may also be used. In addition, while the  
10 receptacles are preferably made entirely from a heat conductive  
11 material, it will be appreciated that only elements of the  
12 receptacle need be made from a heat conductive material. For  
13 example, the receptacle may alternatively include a coiled heating  
14 element which resides in the interior of the receptacle and which  
15 is in contact with the heating element. Furthermore, while the  
16 apparatus has been described using ampoules and reagents and light  
17 wavelengths suitable for testing for total microbial count in a  
18 sample, it will be appreciated that other ampoules testing for  
19 E. coli, Coliforms, and other biological and chemical presences  
20 can also be used. Furthermore, while a preferred incubation  
21 temperature of 34 °C is disclosed, it will be appreciated that  
22 other temperature about 34 °C are suitable, e.g., 32 °C - 37 °C,  
23 for a total microbial test, and that other temperatures may be  
24 preferred for other tests. In addition, while the apparatus has  
25 been described with six independently operable receptacles, the

1 apparatus may include a larger number (e.g., 24 to 36) of  
2 receptacles such that it is suitable for laboratory use or may  
3 include fewer or one receptacle suitable for home or portable use.  
4 It will therefore be appreciated by those skilled in the art that  
5 yet other modifications could be made to the provided invention  
6 without deviating from its spirit and scope as claimed.